Determination of Irradiation D-Values for *Aeromonas hydrophila*

ABSTRACT

This study examined the radiation resistance of *Aeromonas hydrophila,* a psychrotrophic pathogen of emerging importance. Five strains of *Aeromonas hydrophila* (three clinical and two food isolates) were irradiated in a Cesium-137 source at doses up to 150 Krads. The bacterium was irradiated in growth broth, phosphate buffer, ground bluefish or ground beef. Surviving bacteria were counted on nutrient agar or starch ampicillin agar. Radiation resistance was expressed as D-values (dose in Krads to yield a 10-fold decrease in viable number) and ranged from 14 to 22 Krads at 2 ± 1°C for most variables studied. Decreasing the temperature during irradiation increased the radiation resistance (raised the D-values). The results of this study indicate that a pasteurizing dose of ionizing radiation of 150 Krads is sufficient to kill the levels of *Aeromonas hydrophila* found in retail fresh foods.

Aeromonas hydrophila is becoming recognized as a major cause of diarrhea in humans (*2-4,6*). Recent work from this laboratory (*13*) has indicated that clinical isolates of *A. hydrophila* are readily capable of growth at refrigeration temperatures (4 to 5°C). In addition, a survey (*14*) of retail foods, such as fish, seafood (shrimp, scallops and oysters), red meat, poultry and raw milk, indicated that *A. hydrophila* was present in virtually all foods sampled, often at levels of 10^5/g or ml of food.

Radiation processing has been proposed as an alternative method for maintaining the microbiological shelf life of various fresh foods, including fishery products. Giddings (*5*) has discussed four applications of ionizing energy for processing of fishery products: (a) radurizing (pasteurization of certain fresh finfish and shellfish -- 75 to 250 Krads), (b) radicidation (sanitization of frozen products -- 250 to 1000 Krads), (c) destruction of insect eggs and larvae (in dry fish -- doses below 100 Krads), and (d) radiation sterilization (radappertization, non-refrigerated long-term shelf life, a 12 D process -- 3 to 4 Mrads).

Mossel and Van Netten (*12*) have suggested that radiation can be used on foods of animal origin to eliminate enteric pathogens they may contain.

The purpose of this study was to determine the radiation resistance of *A. hydrophila* in growth medium, phosphate buffer, and ground fish and beef, and to determine the effect of growth phase and temperature on this radiation resistance.

MATERIALS AND METHODS

Organisms

Five strains of *A. hydrophila* were used in these studies, i.e., three clinical isolates (BW 83, BA2 and K144) and two food isolates, B-2-10 (from ground beef) and F-6-10 (from fish), isolated in our laboratory.

Growth of cultures

The cultures were grown in brain heart infusion (BHI) broth (Difco) at 28°C with agitation (200 rpm) for either 3 h (log phase cells) or 24 h (stationary phase cells). After growth, the cultures were either irradiated directly in the growth broth or centrifuged (16,300 × *g*) for 10 min at 5°C and resuspended in either potassium phosphate buffer (pH 7.2, 0.1 M), ground bluefish or commercial ground beef (23.1% fat by standard AOAC methodology), and then irradiated.

Irradiation

All material (liquids and foods) were irradiated in a Cesium-137 source (dose rate of ca. 10 Krads/min) at doses up to 150 Krads. Except when the specific effect of temperature was studied, all materials were irradiated at 2 ± 1°C. To study the effect of temperature on radiation destruction of *A. hydrophila,* the inoculated bluefish samples were irradiated at 22°C, -15 ± 2°C or 2 ± 1°C. The liquid cultures were irradiated in 5-ml amounts in sterile 16-ml polypropylene culture tubes (100 × 17 mm, Falcon 2018). The bluefish and ground beef were irradiated in 20-g amounts in Stomacher 400 bags (Cooke Laboratory Products, Alexandria, VA).

Dosimetry

The doses of irradiation received by each sample (liquid or food) were determined by a ferrous-cupric dosimetry solution (*8*).

Survivors

The number of cells surviving various doses of radiation was

determined by surface plating appropriate dilutions (made in 0.1% peptone water) onto duplicate nutrient agar (Difco) or starch ampicillin agar (*14*) plates. Colonies were counted after 24 h at 28°C.

Data on D-values

Survivor plots ($\log_{10}$ No. of survivors vs. dose) were determined for each variable by regression analysis of the data. Correlation coefficients ≥ 0.96 were obtained for all strains and variables. Decimal reduction doses (Krads to yield a 10-fold decrease in viable cell count) were calculated as the reciprocal of the slope obtained from regression analysis.

RESULTS

The susceptibility of *A. hydrophila* to irradiation was initially assessed in growth broth and phosphate buffer. Survivors were plated on both nutrient agar and starch ampicillin agar, to determine if the latter was suitable for detecting survivors in foods. These data are presented in Table 1. The data indicate that the D-values are similar whether the organisms are irradiated in buffer or growth medium, and whether the survivors are determined on nutrient agar or starch ampicillin agar. When the raw data for all four variables were included in the calculation of the D-values for each strain, D-values equivalent to the average of the four D-values were obtained. A combined correlation of ≥ 0.96 was also obtained for each strain for the four variables.

The effect of culture age (growth phase) on D-values was studied (Table 2). No significant differences in radiation sensitivity were observed among log and stationary phase cells.

The data in Tables 1 and 2 represent D-values obtained when the organism was irradiated at 2°C in either the growth broth or phosphate buffer. Because *A. hydrophila* was detected in a wide range of commercial fish and meat samples purchased at retail markets (*14*), it was of interest to determine the radiation resistance of the organism in representative food products. Radiation D-values for *A. hydrophila* in bluefish are given in Table 3. Temperatures of irradiation were: room temperature (22 ± 1°C), ice bath (2 ± 1°C), and frozen (-15 ± 2°C). As anticipated, the D-values increased with decreasing temperature of irradiation. When *A. hydrophila* was irradiated in ground beef, D-values comparable with those obtained when the organism was irradiated in growth broth, buffer and bluefish were observed (Table 4).

DISCUSSION

The radiation D-values for *A. hydrophila* presented here provide previously unavailable data for this emerging psychrotrophic foodborne pathogen. The D-values given in Tables 1 to 4 indicate that this bacterium is relatively sensitive to radiation. The radiation D-values for *A. hydrophila* (Tables 1 to 4) are comparable with those determined by Tarkowski et al. (*15*) for *Yersinia enterocolitica* and *Campylobacter jejuni*, and less than the values for *Salmonella* (55 to 78 Krads), when these organisms were irradiated in ground beef. Lambert and Maxcy (*9*) also reported D-values for *C. jejuni* of 16.1 Krads in ground beef and 18.6 Krads in ground turkey. Further, the D-values for *A. hydrophila* are similar to those reported for other gram-negative bacteria (*7*).

The effect of temperature on radiation killing of *A. hydrophila* (Table 3) is comparable to this effect reported for other bacteria, i.e., low temperature of irradiation increases the D-values (at lower temperatures, the cells be-

TABLE 1. *Effect of plating medium and irradiation medium on D-values of five strains* A. hydrophila. *(temp. of irradiation 2 ± 1°C).*

	Plating medium			
	Starch ampicillin agar		Nutrient agar	
	Irradiation medium			
Strain	Growth medium	Phosphate buffer	Growth medium	Phosphate buffer
K144	16.2[a]	15.5	15.8	14.8
BA2	18.7	18.1	18.8	18.6
BW83	16.8	15.9	15.7	15.6
F6-10	15.7	15.7	15.5	14.0
B2-10	15.5	13.7	15.4	14.9

[a]D-values in Krads.

TABLE 2. *Effect of growth phase on D-values of five strains of* A. hydrophila *(irradiated in culture broth and plated on nutrient agar, temperature of irradiation 2 ± 1°C).*

Strain	Stationary-phase cells	Log-phase cells
K144	18.1[a]	18.0
BA2	19.0	19.5
BW83	16.5	18.3
F6-10	17.6	16.9
B2-10	17.8	21.6

[a]D-values in Krads.

TABLE 3. *Effect of temperature of irradiation on D-values of five strains of* A. hydrophila *in ground bluefish (plated on starch ampicillin agar).*

	Temperature of irradiation (°C)		
Strain	22±1	2±1	-15±2
K144	13.7[a]	17.7	26.2
BA2	15.2	19.3	31.4
BW83	14.5	16.1	34.0
F6-10	11.0	14.1	23.3
B2-10	11.3	15.6	22.2

[a]D-values in Krads.

TABLE 4. *Radiation D-values of five strains of* A. hydrophila *in ground beef (23.1% fat; temperature of irradiation 2±1°C; plated on starch ampicillin agar).*

Strain	D-value (Krad)
K144	14.0
BA2	14.4
BW83	18.9
F6-10	15.1
B2-10	15.0

come more resistant). This increasing resistance as the temperature of irradiation is lowered has been observed for *C. jejuni (9), Salmonella typhimurium (10), Streptococcus faecium* R53, *Pseudomonas* spp. and *Alcaligenes* spp. (*11*), and *S. faecium* α 21 (*1*). Igram and Farkas (*7*) have stated that for vegetative cells, radiation resistance about doubles when the cells are irradiated frozen. The results presented here (Table 3) support this for *A. hydrophila*.

The results of this study indicate that over the dose ranges used (up to 150 Krads), the killing plots were linear, i.e., the correlation coefficient between $\log_{10}$ viable *A. hydrophila* vs. dose were ≥ 0.96. In these experiments, the viable counts ranged from 10^9 to 10^2/g or ml. These linear dose response plots for *A. hydrophila* are in contrast to those observed for *S. faecium* α 21 (*1*), *S. typhimurium* (*10*), *Escherichia coli* and *Alcaligenes* spp. (*11*) in which plots representing populations of mixed resistance or with clumping (colony-forming units) were observed.

Similar D-values were obtained for stationary and log-phase cells of *A. hydrophila*. This is comparable to the observations of Lambert and Maxcy (*9*) for *C. jeuni*.

Except for the temperature effects, the D-values observed for *A. hydrophila* ranged from 14 to 22 Krads. In a previous study (*14*), the prevalence of *A. hydrophila* in fresh fish, red meat and poultry samples obtained at retail outlets was 10^2 to 10^5/g. A dose of 125 to 150 Krads, as used in these experiments, would be sufficient to eliminate this organism from food.

ACKNOWLEDGMENTS

Reference to brand or firm name does not constitute endorsement of the U.S. Department of Agriculture over others of a similar nature not mentioned.

REFERENCES

1. Anellis, A., D. Berkowitz, and D. Kemper. 1973. Comparative resistance of nonsporogenic bacteria to low-temperature gamma radiation. Appl. Microbiol. 25:517-523.
2. Buchanan, R. L. 1984. The "new" pathogens: an update of selected examples. Assoc. Food Drug Off. Quart. Bull. 48:142-155.
3. Buchanan, R. L., and S. A. Palumbo. 1985. *Aeromonas hydrophila* and *Aeromonas sobria* as potential food poisoning species: a review. J. Food Safety 7:15-29.
4. Escheverria, P., R. B. Sack, N. R. Blacklow, P. Bodhidatta, B. Rowe, and A. McFarland. 1984. Prophylactic doxycycline for travelers' diarrhea in Thialand. Further supportive evidence of *Aeromonas hydrophila* as an enteric pathogen. Am. J. Epidemiol. 120:912-921.
5. Giddings, G. G. 1984. Radiation processing of fishery products. Food Technol. 38(4):61-65, 94-97.
6. Hazen, T. C., C. B. Fliermans, R. P. Hirsch, and G. W. Esch. 1978. Prevalence and distribution of *Aeromonas hydrophila* in the United States. Appl. Environ. Microbiol. 33:114-122.
7. Ingram, M., and J. Farkas. 1977. Microbiology of food pasteurized by ionizing radiation. Acta Aliment. 6(2):123-185.
8. Jarrett, R. D., Jr. 1967. Radiation dosimetry in relation to high intensity radiation sources. Adv. Chem. 65:78-86.
9. Lambert, J. D., and R. B. Maxcy. 1984. Effect of gamma radiation on *Campylobacter jejuni*. J. Food Sci. 49:665-667, 674.
10. Licciardello, J. J. 1964. Effect of temperature on radiosensitivity of *Salmonella typhimurium*. Food Res. 29:469-474.
11. Matsuyama, A., M. J. Thornley, and M. Ingram. 1964. The effect of freezing on the radiation sensitivity of vegetative bacteria. J. Appl. Bacteriol. 27:110-124.
12. Mossel, D. A. A., and P. Van Netten. 1982. Whither protection of the consumer against enteropathogenic bacteria on fresh meats and poultry by processing for safety. pp. 2-19. *In* Food irradiation now. Martinus Nijhoff/Dr. W. Junk Publishers, The Hague, The Netherlands.
13. Palumbo, S. A., D. R. Morgan, and R. L. Buchanan. 1985. The influence of temperature, NaCl and pH on the growth of *Aeromonas hydrophila*. J. Food Sci. 50;1417-1421.
14. Palumbo, S. A., F. Maxino, A. W. Williams, R. L. Buchanan, and D. W. Thayer. 1985. Starch ampicillin agar for the quantitiative detection of *Aeromonas hydrophila*. Appl. Environ. Microbiol. 50:1027-1030.
15. Tarkowski, J. A., S. C. C. Stoffer, R. R. Beumar, and E. H. Kampelmacher. 1984. Low dose gamma irradiation of raw meat. I. Bacteriological and sensory quality effects in artificially contaminated samples. Int. J. Food Microbiol. 1:13-23.